PATENT ABSTRACTS OF JAPAN

(11)Publication number : 05-229948
(43)Date of publication of application : 07.09.1993

(51)Int.Cl. A61K 31/555
A61K 35/74
C12P 17/18
// C07D487/22
(C12P 17/18
C12R 1:06)

(21)Application number : 04-150980
(22)Date of filing : 10.06.1992

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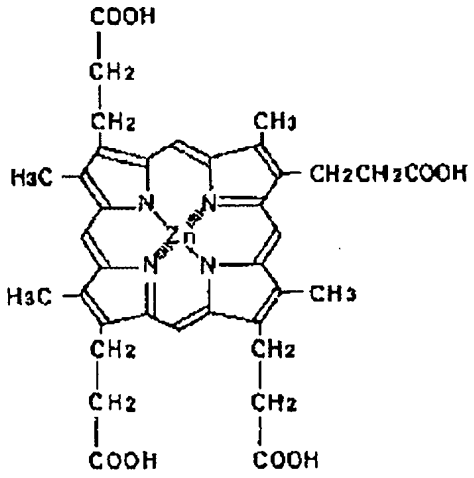
(30)Priority
Priority number : 40334367 Priority date : 25.12.1991 Priority country : JP

(54) THERAPEUTIC AGENT OR DIAGNOSTIC AGNET FOR MALIGNANT TUMOR AND PRODUCTION OF SUBSTANCE AC8007

(57)Abstract:
PURPOSE: To obtain a substance AC8007 useful as a therapeutic agent having properties as a photosensitizer for photochemotherapy or as a diagnostic agent for malignant tumors.
CONSTITUTION: The objective therapeutic agent or diagnostic agent for malignant tumors comprises a substance AC8007, having an estimated chemical structural formula expressed by the formula and the following physico-chemical properties or its nontoxic salt as an active ingredient: (1) Elementary analytical value; C, 60%; H, 5%; N, 8% and Zn, 8-10%. (2) Mass spectrometric value; 717 [MH+, according to the fast atom bombardment-mass spectrometry (FAB- MS)]. (3) Molecular formula; C36H36O8N4Zn. (4) Solubility in solvents; soluble in methanol, ethyl acetate, acetic acid and dimethyl sulfoxide and insoluble in water, hexane and benzene. (5) Color reaction; positive to the potassium permanganate reaction and iodine reaction and negative to ferric chloride reaction, the Dragendorff reaction and ninhydrin reaction. This compound is obtained by culturing a microorganism, belonging to the genus Arthrobacter and capable of producing the substance AC8007, e.g. Arthrobacter.sp. TM-1 (FERM BP-3646) in a culture medium and then collecting the substance from the resultant culture.



LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

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[Patent number]

[Date of registration]

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CLAIMS

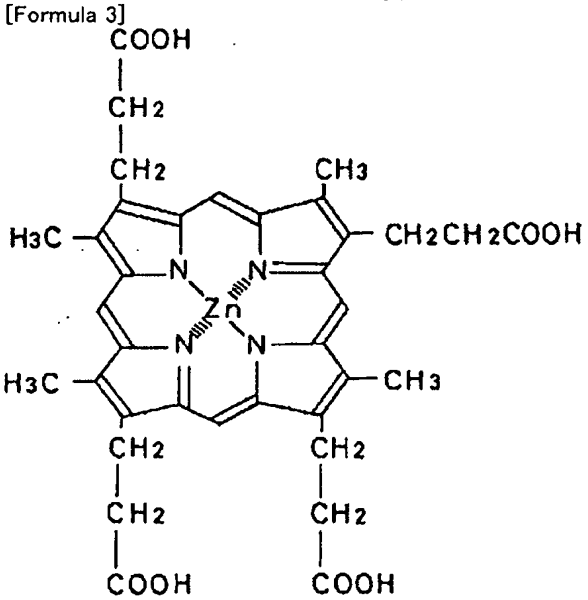
[Claim(s)]
[Claim 1] The therapy agent or diagnostic agent of a malignant tumor characterized by making into an active principle AC8007 matter which has the following physicochemical properties, or its nontoxic salt.
(1) elemental-analysis value C: -- about 60% and H: -- about 5% and N: -- about 8% and Zn:about 8 - 10% mass-analysis [(2)] value 717 (based on MH+ and FAB-MS)
(3) Molecular formula C36H36O8 N4 Zn(4) visible-region absorption spectrum [** 1]

$\lambda_{max}^{メタノール}$ nm ($E_{1cm}^{1\%}$):

[Formula 2] which has absorption characteristic 386 (shoulder) (750), 406 (3525), 538 (185), and near 574(190) nm at least

$\lambda_{max}^{0.1NHCl・メタノール}$ nm ($E_{1cm}^{1\%}$):

(5) infrared absorption spectrums which have absorption characteristic 386 (shoulder) (1275), 402 (4690), 560 (175), and near 591 (60) nm at least (KBr law)
at least -- 3420 -- 2920 -- 1705 -- 1400 -- 1275 -- 1130 -- 940 -- 835 -- cm - one -- the neighborhood -- being characteristic -- absorption -- having -- (-- six --) -- a solvent -- receiving -- solubility -- a methanol -- ethyl acetate -- an acetic acid -- dimethyl sulfoxide -- fusibility, water, a hexane, and benzene -- insoluble (7) color-reaction potassium permanganate reaction and an iodine reaction -- a positivity, a ferric chloride method, a DORAGENDORUFU reaction, and ninhydrin reaction -- electronegative (8) basicity, acidity, and the color low red heat [claim 2] of the neutral distinction acid (9) matter The therapy agent or diagnostic agent of a malignant tumor according to claim 1 to which AC8007 matter is characterized by having the following presumed chemical structure type.
[Formula 3]



[Claim 3] The manufacturing method of AC8007 matter characterized by cultivating AC8007 matter-production bacillus belonging to the Arthrobacter group to a culture medium, and subsequently extracting AC8007 matter from a culture.
[Claim 4] The manufacturing method according to claim 3 whose AC8007 matter-production bacilli belonging to the Arthrobacter group are Arthrobacter ESUPI and TM-1 (FERM BP-3676).

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the manufacturing method of the therapy agent of the malignant tumor which makes an active principle AC8007 matter or its nontoxic salt or a diagnostic agent, and AC8007 matter.
[0002]
[Description of the Prior Art] The photochemical therapy (Photodynamic therapy:PDT) to a malignant tumor is developed, dozens of years pass, many effective examples are checked, and it is used as the radical cure therapy or diagnostic agent of an early malignant tumor. The typical photosensitizer used for this PDT is a porphyrin derivative.
[0003] However, for a porphyrin derivative, the following faults are **. (1) chemical -- the good long wave of (2) organization permeability which is not single -- it is given to (3) neoplasm tissues which do not have absorption in a field that the amount yield of (5) photochemical reaction with the slow elimination from (4) normal cells which is not held for a long time is low etc.
[0004]
[Problem(s) to be Solved by the Invention] It is expected that development of the photosensitizer which can solve at least the one above problem is effective in future PDT. And they are ** ****** because of a malignant tumor therapy of a new and useful photosensitizer with few side effects. The purpose of this invention is to offer the manufacturing method of the therapy agent of the malignant tumor which makes an active principle AC8007 matter or its nontoxic salt or a diagnostic agent, and AC8007 matter.
[0005]
[Means for Solving the Problem] All the photosensitizers in old PDT are the matter compounded chemically, and a problem is ****** in the field of a side effect. From from, this invention persons extracted the matter identified AC8007 matter (refer to JP,2-234688,A) from the culture filtrate of Arthrobacter ESUPI and TM-1 (FERM BP No. 3676) among the natural physiological active substances which a microorganism produces, and this matter found out that it was the matter which has zinc. Furthermore, this invention persons completed the header for this matter being useful as a diagnostic agent as a therapy agent which has a property as a feeling agent of light of PDT of a malignant tumor, and completed a header and this invention for the good manufacturing method of AC8007 matter further.
[0006] AC8007 matter which is effective matter of this invention has a physicochemical property as shown below at least.
(1) elemental-analysis C: -- about 60% and H: -- about 5% and N: -- about 8% and Zn:about 8 - 10% (2) mass analysis 717 (based on MH+ and FAB-MS)
(3) Molecular formula C38H36O8 N4 Zn(4) visible-region absorption spectrum : drawing 1 (neutrality condition), drawing 2 (acid conditions)
[0007]
[Formula 4]

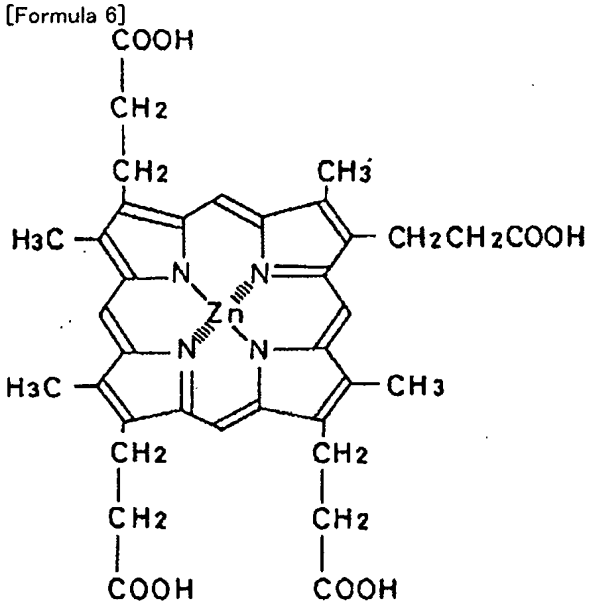
$\lambda_{max}^{メタノール}$ nm ($E_{1cm}^{1\%}$):

[0008] which has absorption characteristic 386 (shoulder) (750), 406 (3525), 538 (185), and near 574(190) nm at least
[Formula 5]

$\lambda_{max}^{0.1NHCl\cdot メタノール}$ nm ($E_{1cm}^{1\%}$):

It has absorption characteristic 386 (shoulder) (1275), 402 (4690), 560 (175), and near 591 (60) nm at least.
[0009] (5) infrared-absorption-spectrum (KBr law): -- drawing 3 -- even if few, it has absorption characteristic of 3420, 2920, 1705, 1400, 1275, 1130, and the 940 or 835cm-1 neighborhood.
(6) It is insoluble [0010] to fusibility, water, a hexane, and benzene in the soluble methanol to a solvent, ethyl acetate, an acetic acid, and dimethyl sulfoxide. (7) a color reaction potassium permanganate reaction and an iodine reaction -- a positivity, a ferric chloride method, a DORAGENDORUFU reaction, and ninhydrin reaction -- color low-red-heat (10) 1 H-NMR(it measures in 400MHz, 27-degree-C, and d6 DMSO): drawing 4 [0011 of electronegative (8) basicity, acidity, and the neutral distinction acid (9) matter --] (11) 13 C-NMR (it measures in 100MHz, 27 degrees C, and DMSO) : the signal shown below at least was accepted. 174.20 (s), 147.62 (s), 147.54 (s), 146.83 (s), 146.77 (s), 146.73 (s), 139.44 (s), 139.30 (s), 136.65 (s), 136.54 (s), 97.06 (d), 96.95 (d), 37.41 (t), 21.63 (t), 21.59 (t), 11.46 (q)
[0012] (12) Thin-layer chromatography (Tokyo formation shrine make, spot film silica gel f use)
Rf=0.45[expansion solvent: Chloroform-methanol acetic-acid (10:1. 5:0.1)]
Rf=0.37[expansion solvent: Butanol-ethanol-chloroform-aqueous ammonia (4:5:2:4)]
[0013] It is presumed that it is structure expressed with a degree type since AC8007 matter has the above property.
[0014]

[Formula 6]



[0015] In order to produce AC8007 matter which is the active principle of this invention, the fermenting method by culture of a microorganism is the most suitable. As a suitable microorganism for production, it is Arthrobacter ESUPI. TM-1 (Arthrobacter sp.TM-1: FERM BP No. 3676) can be mentioned. Bacteria is the bacteria TM-1 share separated from the soil of the Chinese cabbage field of Koka, Koka-gun, Shiga-ken-cho, is an example of the strain used the most effective in this invention, and when it shows the mycology-property of a bacteria stock, it is as follows.
[0016] In addition, the identification trial was carried out in identification of a bacteria stock according to "guidance of medicine bacteria identification, the 2nd edition, 1974", "Microbiological Methods 3 volume", etc. The experimental result was identified as contrasted with "guidance of medicine bacteria identification, the 2nd edition, 1974", "Bergey's Mannual of Systematic Bacteriology Vol.1 (1984), Vol.2 (1986), Vol.3" (1989), etc. Culture temperature was performed at 28-30 degrees C. (+: -- there is no publication in a positivity, a (+):weak positivity, -:negative, NT:un-examining, and ND:reference -- NC:change of is not done)

[0017] The description ordinary agar slant-medium circumference of experimental result 1. growth forms a round notch-like cluster, and a center rises to convex. Although it is translucent and humid and off-white - light ocher is presented, soluble pigment does not produce.
Although ordinary agar flat-surface culture-medium growth is bad, it is grown to a line. Although it is translucent and humid and off-white - light ocher is presented, soluble pigment does not produce.
Liquid medium (peptone water)
It becomes muddy uniformly.
It becomes Ritmos milk culture-medium alkali, and peptonizates.
[0018] 2. GCmol %NT3. of DNA -- a long rod is shown in the first half of the description culture of a main isoprenoid quinone NT4. gestalt, it curves and magnitude has some which show the shape of V character by 0.8x4-5 micrometers. The bacteria which change at a culture anaphase a 1.2x1.5-micrometer short rod - in the shape of a ball.
[0019]
5. Physiology - Chemical -- Description -- A Gram's stain + A KOH reaction - Acid fast stain - Capsule formation - [0020]
The OF test (Hugh-Leifson) NT The OF test (it is NH4 H2 PO4 to the source of N) O Growth by aerobic + Growth by the aversion + Growth temperature 42 degrees C - 37 degrees C +20 degree C + 10 degrees C NT [0021]
Salt resistance 0% + 0.5% + 3.0% NT 5.0% NT Growth pH 4.7- 5.6 +9.0 + 10.0 -[0022]
Gelatin decomposition - The amylolysis - Casein decomposition + Esculin decomposition NT Cellulose decomposition - Thyrosin decomposition NT Tween80 decomposition NT Arginine decomposition NT Catalase production - oxidase production NT [0023]
Lecithinase production - Urease production (SSR) NT Urease production (Chris.) NT The Indore production - Hydrogen-sulfide production (lead acetate paper) + Acetoin production (K2 HPO4) - Acetoin production (NaCl) - MR test - Nitrate reduction test (aerosis) - (detection of NO2-) - (detection of NO3-) + [0024]
The availability in the Simmonds culture medium (alkali production) Citrate - Malate + A maleate - Malonic-acid salt - Propionate - Gluconate (+)
Succinate + [0025]
The availability in a coulisse TENZEN culture medium (alkali production) Citrate + Malate + A maleate + Malonic-acid salt + Propionate + Gluconate + [0026]
Succinate + From a glucose, production of gas - From sugar, production of an acid (it is NH4 H2 PO4 to a nitrogen source) Ribitol - L(+)-arabinose - Cellobiose - Dulcitol - MERI-erythritol - Fructose + [0027]
D-galactose - D-glucose + A glycerol + An inositol - An inulin - Lactose - maltose - Mannitol -[0028]
A mannose - MEREJITOSU - A melibiose - A raffinose - L(+)-rhamnose - D-ribose - salicin - L-sorbose - sorbitol - [0029]
Starch - Saccharose + Trehalose (+)
D-xylose + [0030] 6. Other Analysis (Chemical Analysis Etc.)
bacteria stock TM-1 -- main -- description -- the bacteria of a gram positive show a rod in short-time culture, and the cell of a stationary phase becomes a spherical - short rod. Moreover, the cell of V and a Y shape is also seen. Motile nothing one,

catalase un-producing, and a glucose are decomposed in oxidation, and an acid is produced.
[0031] The fungus which changes from a rod-like cell to a capitulum with the identification gram-positive bacteria of bacteria stock TM-1, and shows the array of V, a Y shape (pseudo-branching), etc. has the Arthrobacter group of a Coryneform group. a bacteria stock -- main -- it was judged as what belongs to an Arthrobacter group, judging from description (there was no publication of the fungus which corresponds although other Coryneform groups were searched).
[0032] bacteria stock TM-1 -- many -- each strain of description and an Arthrobacter group -- many -- although, as for the acid production pattern from sugar, A.simplex was alike as a result of contrasting description, the description of growth by the resolution, the catalase production ability, and the Ritmos milk culture medium of starch was not in agreement. Therefore, it is Arthrobacter ESUPI about a bacteria stock. Identification naming was carried out with TM-1 (Arthrobacter sp.TM-1). Bacteria and Arthrobacter ESUPI (Arthrobacter sp.) TM-1 share were deposited with the Fermentation Research Institute, the Agency of Industrial Science and Technology, (FERM BP No. 3676, FERM BP-3676).
[0033] In order to obtain effective product AC8007 matter of this invention, the variant or variety which has the capacity that AC8007 matter can be produced is first cultivated aerobically in a culture medium according to a conventional method in the amount which can extract the above-mentioned microorganism or AC8007 matter. As a culture medium illustrated by this invention, AC8007 matter-production bacillus belonging to the above-mentioned Arthrobacter group 1l. of ion-exchange pure water It hits. Isopropyl alcohol 10ml, 0.3g of yeast extracts, Peptone 3.0g, 3.0g of ammonium nitrates, 0.4g of potassium phosphate, 1.5g of phosphoric-acid disodium, 0.5g of magnesium sulfate, 10mg of manganese sulfates, What is necessary is to carry out inoculation to the 500ml Erlenmeyer flask which held 100ml of sterilized culture media containing 10mg of zinc sulfates, copper-nitrate 50microg, molybdenum-trioxide 10microg, and 5.0g of calcium carbonates, and just to carry out shaking culture for three days at 30 degrees C. 1ml inoculation of this culture is carried out to the 500ml Erlenmeyer flask containing 100ml of the same culture media as the above, and it should just carry out shaking culture to it for five days at 30 degrees C.
[0034] Thus, what is necessary is to filter a culture, to add a nonaqueous solubility organic solvent, for example, ethyl acetate, a butanol, butyl acetate, etc. to the filtrate, to extract with Acidity pH, to **** with Alkali pH subsequently to water, and just to carry out solvent extraction with Acidity pH further, since AC8007 matter mainly exists in culture filtrate in order to extract AC8007 matter from the obtained culture. This is further given to the chromatography by silica gel, the alumina, a synthetic adsorbent, etc., separation generation can be carried out or separation acquisition can also be carried out using high performance chromatography etc. Moreover, AC8007 obtained matter can also be made into salts, such as alkaline-earth-metal salts, such as alkali-metal salts, such as sodium salt, a calcium salt, and magnesium salt, ammonium salt, and a salt with a well-known nontoxic organic amine, by the well-known approach. Thus, AC8007 obtained matter has a physicochemical property which was described above. AC8007 matter of this invention has the operation shown below.
[0035] (1) 0.05ml intracutaneous vaccination of sarcoma-180 (1x108 cells/ml) was carried out ICR mouse 20g (male) behind [one] five photosensitization curative effect groups to antitumor action 1Sarcoma-180. Two days after, AC8007 matter and hematoporphyrin (HpD; sigma company make) were dissolved in the physiological saline which added the 3ml 0.1mM tris hydrochloric-acid buffer solution (pH7.4) for 15mg, respectively, and it medicated mouse intraperitoneal with 0.2ml.
[0036] It is ****** so that heat may not get across an optical exposure to a tumor site for 10 minutes by RUMINA ace L-150S (product made from a wood clock) which performed pentobarbital anesthesia after 10 minutes and used the halogen lamp (JR15V150WB) for the pan after 10 minutes. The major axis and minor axis of a neoplasm were measured from the 3rd, and the value calculated based on the following formulas was made into the magnitude of a neoplasm.

$$\text{腫瘍の大きさ} = \frac{\text{長い径 (mm)} \times \text{短径 (mm)}}{2}$$

After 50 mg/kg intraperitoneal administration, result AC8007 matter controlled prominent growth of sarcoma-180 as by irradiating light in 20 minutes showed it to Table 1 and drawing 5 .
[0037]
[Table 1]

	腫瘍の大きさ (mm)					
	日数 (日)	3	4	5	6	7	8
対照	非照射	20.1	26.0	35.6	48.3	56.3	52.9
	照射	21.5	27.0	36.0	48.0	58.2	58.1
AC8007物質 50mg/kg	非照射	21.1	28.2	38.1	46.6	57.6	58.1
	照射	21.3	10.6	14.6	17.3	23.9	23.7
ヘマトポルフィリン50mg/kg	非照射	21.0	26.6	37.1	45.6	53.0	53.0
	照射	20.6	17.6	23.3	31.9	60.0	45.5

[0038] 2) By the same approach as the operation above 1 to Sarcoma-180, the ICR mouse was used one groups [three]. 5mg / 2ml, 2.5mg / 2ml, and 1.25mg / 2ml solution were prepared with the physiological saline which added the 0.1M tris hydrochloric-acid buffer solution (pH7.4) so that it might become 25 mg/kg, 12.5 mg/kg, and 6.3 mg/kg, respectively about AC8007 matter,

hematoporphyrin dihydrochloride (NO.H-1875, about 75% of purity, sigma company make), and hematoporphyrin (NO.H-5518, about 50% of purity, sigma company make). Intraperitoneal [of a mouse] is medicated with 0.2ml of this mixed solution, and it is ****** about a curative effect. They are the hematoporphyrin which a result is as being shown in Table 2 and drawing 6 , and used AC8007 matter as a control drug and hematoporphyrin 2, and HCl. It was checked that effectiveness is excellent.
[0039]
[Table 2]

	投与量	腫瘍の大きさ(㎟)				
		日数				
	(mg/kg)	3	4	5	6	7
対照	0		18.7	22.7	34.1	36.7
AC800 7物質	25		7.8	10.5	14.5	17.6
	12.5		11.3	14.4	21.0	23.7
	6.3		19.3	20.9	30.9	34.4
ヘマトポルフィリン 2HCl	25	12.0	12.8	15.3	24.2	25.1
	12.5		12.9	16.3	25.6	32.0
	6.3		17.4	24.6	35.9	35.8
ヘマトポルフィリン	25		9.7	12.5	20.8	20.0
	12.5		16.1	18.0	28.4	28.9
	6.3		18.1	20.7	39.0	36.7

[0040] (2) 0.05ml intracutaneous vaccination of Sarcoma-180 (1x108 cells/ml) was carried out behind [one] the application ICR mouse to tumor diagnosis. If AC8007 matter 50 mg/kg administration is carried out and it irradiates through lightguide two days after, a neoplasm can perform partial decision according to fluorescence.
[0041] (3) BDF1 of three photosensitization curative effect groups to B-antitumor action 1 16 melanoma 0.05ml intracutaneous vaccination of the B-16 melanoma (2x107 cells/ml) was carried out mouse 20g (male) back [one]. 10mg / 2ml, 5mg / 2ml, and 2.5mg / 2ml solution were dissolved in the physiological saline which added the 0.1M tris hydrochloric-acid buffer solution (pH7.4) so that it might become 50 mg/kg, 25 mg/kg, and 12.5 mg/kg about AC8007 matter and hematoporphyrin (HpD; sigma company make) seven days after, respectively, and it medicated mouse intraperitoneal with 0.2ml. Pentobarbital anesthesia was performed after 10 minutes, and it carried out so that heat might not get across an optical exposure to a tumor site for 10 minutes by RUMINA ace L-150S (product made from a wood clock) which used the halogen lamp (JR15V150WB) for the pan after 10 minutes. The major axis and minor axis of a neoplasm were measured from the 8th, and the value calculated based on the following formulas was made into the magnitude of a neoplasm.

$$\text{腫瘍の大きさ} = \frac{\text{長径 (㎜)} \times \text{短径 (㎜)}}{2}$$

[0042] After 50 mg/kg and 25 mg/kg intraperitoneal administration, result AC8007 matter controlled growth of B-16 prominent melanoma as by irradiating light in 20 minutes showed it to Table 3 and drawing 8 , and the photosensitization therapy by the optical exposure of HpD was shown in Table 3 and drawing 9 .
[0043]
[Table 3]

	投与量	腫瘍の大きさ(mm²)					
		日数					
	(mg/kg)	7	8	9	10	11	12
対照	0	11.9	15.9	22.6	27.04	27.46	40.8
AC800	50	11.7	6.9	13.1	14.4	18.2	20.2
7物質	25	11.5	10.9	14.2	19.8	23.1	30.9
	12.5	11.0	12.2	17.4	21.7	28.1	32.5
HpD	50	11.3	11.10	13.3	15.0	18.0	24.9
	25	12.8	11.3	14.6	21.0	25.4	28.5
	12.5	12.1	11.2	17.3	25.2	28.9	43.3

[0044] To the tumorigenesis mouse using Sarcoma-180 of a more than, and B-16 melanoma, a curative effect is accepted by PDT and AC8007 matter is accepted to be effective also to Homo sapiens origin neoplasms, such as Homo sapiens lung origin malignant tumor A549 share, 521 shares of Homo sapiens large intestine origin malignant tumors AZ, and Homo sapiens melanoma G361 share, a Homo sapiens uterine cervix origin Hela cell.
[0045] (3) Even if it carries out 400 mg/kg intraperitoneal injection of the acute toxicity AC8007 matter at a mouse, the example of death is seen, and it is inside ****.
(4) A mouse acute phototoxicity experiment photosensitizer is incorporated by the living body, and causes photosensitivity in direct rays. A symptom starts in the erythema and pain and itch of HIFU at first, an edema occurs after that, and a necrosis of HIFU is observed although several days after swelling pulls. When critical, there is also an example which will be in a coma and dies.
1) Intraperitoneal administration was performed so that it might become an experiment approach ICR (**, 22-25g) mouse (one groups [three]) with 100 mg/kg and 50 mg/kg about each about AC8007 and HpD. The exposure was carried out from the upper part of immediately after administration to a mouse for 2 hours (it maintains on 25-28-degree-C temperature conditions) with the halogen lamp (the RUMINA ace, Wood Clock, JCR15V, 150WB). At this time, it was 32000 luxs. It usually bred after that and mouse weight and life and death were observed.
2) an experiment result -- the result is shown in Table 4.
[0046]
[Table 4]

群		例数	死亡例		
			1日目	2日目	3日目
コントロール	照射	3	0	0	0
AC8007	100 mg/kg (ip) 照射	3	0	0	0
物質	50 mg/kg (ip) 照射	3	0	0	0
HpD	100 mg/kg (ip) 照射	3	3		
	50 mg/kg (ip) 照射	3	1	0	0

[0047] By AC8007 matter (100 mg/kg, 50 mg/kg) administration group, the example of death was not accepted as shown in the above-mentioned table 4. It died from the HpD100 mg/kg administration group by all the example next days, and 1/3 example of death was accepted by 50 mg/kg administration group on the next day. It is as weight change being shown in drawing 10 , and the increment in weight was accepted by AC8007 matter group like the non-prescribed a medicine for the patient exposure control group. It survived by the HpD50 mg/kg administration group, and the loss weight was accepted to following **** and weight change of 2/3 example was recovered 72 hours after on the next day. In acute phototoxicity, AC8007 matter accepted high safety compared with HpD as mentioned above.
[0048] As stated above, AC8007 matter is dissolved in the physiological saline (pH7.4) containing the 0.1M tris hydrochloric-acid buffer solution, by intraperitoneal administration, intravenous administration, internal use, etc., by prescribing this matter for the patient, it goes to a tumor site and light, laser, a supersonic wave, an X-ray, etc. are irradiated at a ********** period.

Consequently, it can close, if a malignant tumor cell is in a necrosis, and growth of a malignant tumor can be controlled. Therefore, it dissolves in the physiological saline which are per [1] adult – 10 mg/kg, and added the sterile buffer solution (pH7.4 neighborhood) as a medication method as a dose of effective matter AC8007 matter of this invention on the 1st, and intravenous administration, partial administration, or internal use performs.

[0049]

[Effect of the Invention] This invention is effective in the therapy and diagnosis of a malignant tumor by a photosensitization and a fluorescence operation of AC8007 matter.

[0050] Example 1(1) Arthrobacter ESUPI and MT-1 (FERM BP-3676) 1l. of ion-exchange pure water It hits. Isopropyl alcohol 10ml, 0.3g of yeast extracts, Peptone 3.0g, 3.0g of ammonium nitrates, 0.4g of potassium phosphate, 1.5g of phosphoric-acid disodium, 0.5g of magnesium sulfate, 10mg of manganese sulfates, Inoculation was carried out to the 500ml Erlenmeyer flask which held 100ml of sterilized culture media containing 10mg of zinc sulfates, copper-sulfate 50microg, molybdenum-trioxide 10microg, and 5.0g of calcium carbonates, and shaking culture was carried out for three days at 30 degrees C. 1ml inoculation of this culture was carried out to the 500ml Erlenmeyer flask containing 100ml of the same culture media as the above, and it carried out shaking culture to it for five days at 30 degrees C. This culture is disinfected according to centrifugal separation, after setting 200 500ml ** flasks, and it is about 19l. of culture supernatants. It obtained.

[0051] (2) It is 8l. of ethyl acetate after adjusting pH to 2.0 with an acetic acid about the culture supernatant liquid obtained above (1). The active principle was extracted. It is 4l. of water to this extract. In addition, it is ****** about the extract operation after aqueous ammonia adjusts pH of a water layer to 9.0. Vacuum concentration of the separated water layer was carried out even to about 500ml. It let concentration liquid pass in the column of 400ml of adsorption resin (diamond ion HP-20, Mitsubishi Kasei Corp. make). 3l. of water After washing, 3l. of water It is ****** about elution by the linear-model concentration gradient reach and using 3l. of 80% acetone water. 2l. of the beginning When it threw away and every 17g fractionation was performed after that, elution of the active principle was carried out to fraction No.87-150. Vacuum concentration of these fractions was collected and carried out, and low-red-heat powder was obtained.

[0052] It charged in the column of the silica gel (the Merck Co. make, Art7734, 350ml) which filled up this powder with the mixed solvent of butanol-ethanol-chloroform-aqueous ammonia (4:5:2:3) beforehand, and was eluted with the same mixed solvent as the above. When 600ml of the beginning was thrown away and every 17g fractionation was performed after that, elution of the active principle was carried out to fraction No.11-40. Vacuum concentration of these fractions was collected and carried out, and the low-red-heat powder of AC8007 matter was obtained.

[0053] (3) The low-red-heat powder obtained above (2) was dissolved in 2ml of mixed solvents of a methanol 50% ammonium acetate water solution (55:45), this was charged in the column of octadecyl silica gel (mountain village chemistry company make, YMC-GEL-ODS, 662ml), and it was eluted with the same mixed solvent as the above. In this, when it performed 20ml of fractionation at a time, elution of the active principle was carried out to fraction No.42-55. These fractions were collected and bottom methanol distilling off of reduced pressure was carried out. It let residue pass in the column of adsorption resin (the Mitsubishi Kasei Corp. make, diamond ion HP-20,100ml). 1l. of water It was eluted with acetone water 80% after washing. Vacuum concentration of the eluate was carried out and residue was dissolved in ethyl acetate.

[0054] After the 10mM ethylenediamine tetra-acetate water solution (pH2) washed this solution, vacuum concentration of the ethyl acetate layer was carried out. AC8007 matter (isolation liquid) refined by collecting the settlings which added the hexane to residue and deposited on a glass filter, and carrying out reduced pressure drying was obtained as low-red-heat powder. Yield of 159mg.

[0055] (4) After dissolving 10mg (isolation liquid) of AC8007 matter in 1ml of 4-N aqueous ammonia above (3), it freeze-dried and the ammonium salt of AC8007 matter was obtained. Yield of 11mg.

[0056] Example sarcoma-180 (1x108 cells/ml) was inoculated behind the ICR mouse of two groups [three], 20g, and a male in 0.05ml hide, and it medicated intraperitoneal with AC8007 matter two days after. It is ****** so that pentobarbital anesthesia may be performed after 10 minutes and heat may not get across an optical exposure to a tumor site for 10 minutes after that further by RUMINA ace L150s (product made from a wood clock) which used the halogen lamp (JCR15V150WB) after 10 minutes. The magnitude of a neoplasm was measured from the 3rd day to the 8th day, and the prominent tumor growth depressant action of AC8007 matter as shown in Table 1 and 2 was accepted.

[0057] Example 3AC8007 matter was dissolved so that it might become a sterile physiological saline (it adjusts to the pH7.5 neighborhood) in ml and 5mg /. Sterile filtration of this was carried out with 0.22-micrometer Millipore filter, and it considered as injections.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the visible-region absorption spectrum of AC8007 matter when using a methanol as a solvent.
[Drawing 2] It is the visible-region absorption spectrum of AC8007 matter when using 0.1NHCl(s) and a methanol as a solvent.
[Drawing 3] It is the infrared absorption spectrum of AC8007 matter.
[Drawing 4] It is the proton nuclear-magnetic-resonance spectrum of AC8007 matter.
[Drawing 5] It is the curve which showed the photosensitization curative effect of AC8007 matter.
[Drawing 6] It is the curve which showed the photosensitization curative effect of AC8007 matter.
[Drawing 7] It is the fluorescence spectrum of AC8007 matter.
[Drawing 8] It is the curve which showed the photosensitization curative effect of AC8007 matter.
[Drawing 9] It is the curve which showed the photosensitization curative effect of the hematoporphyrin (HpD) as contrast.
[Drawing 10] It is the curve of the weight change in the mouse acute phototoxicity experiment of AC8007 matter and hematoporphyrin (HpD).

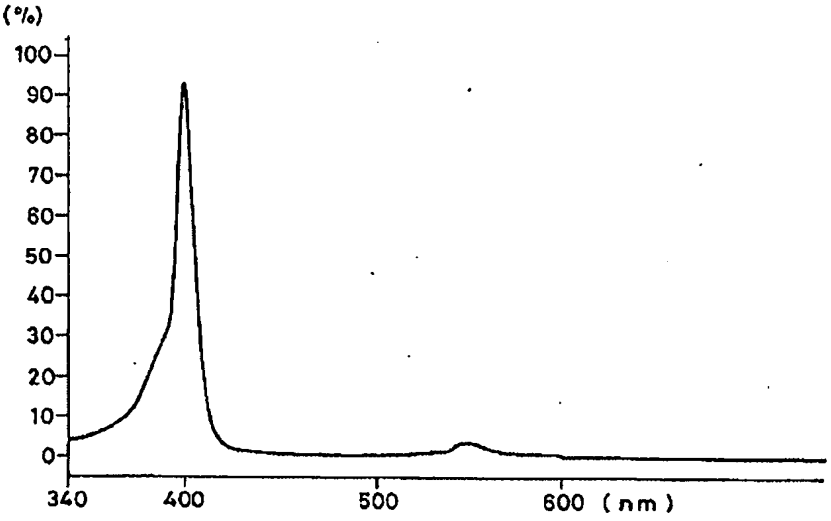
[Translation done.]

* NOTICES *

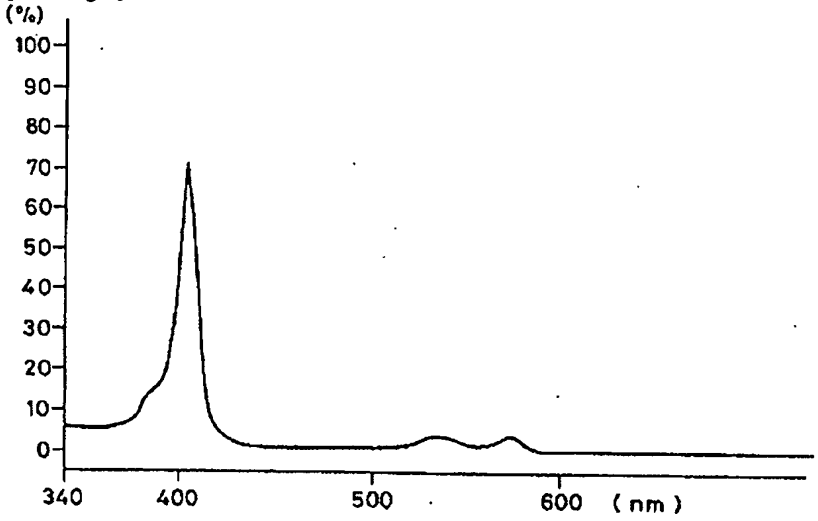
1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

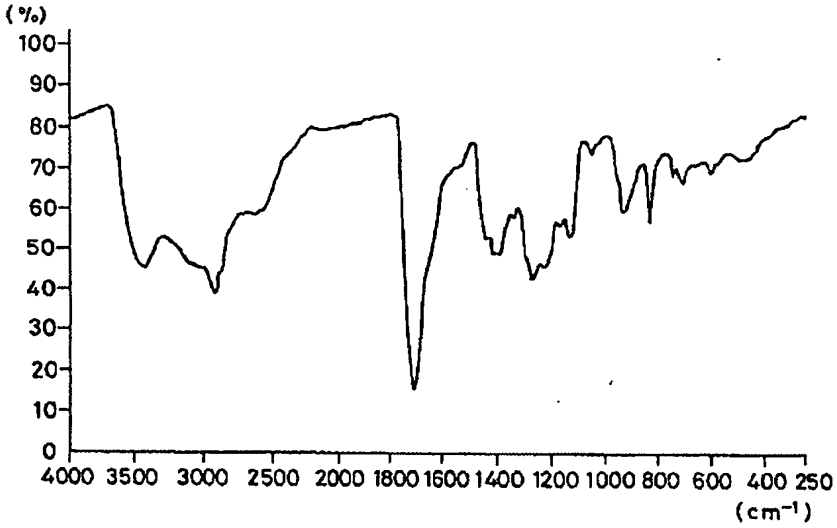
[Drawing 1]



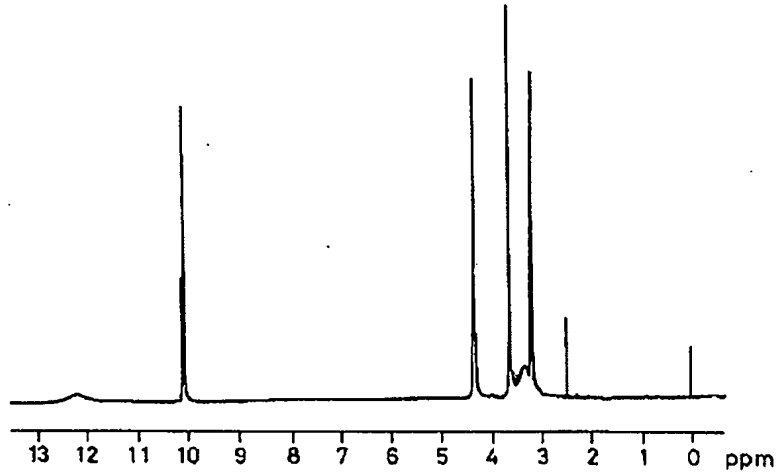
[Drawing 2]



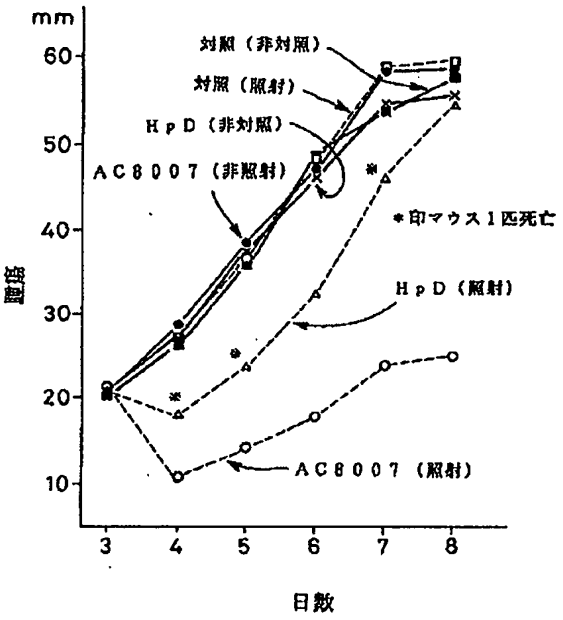
[Drawing 3]



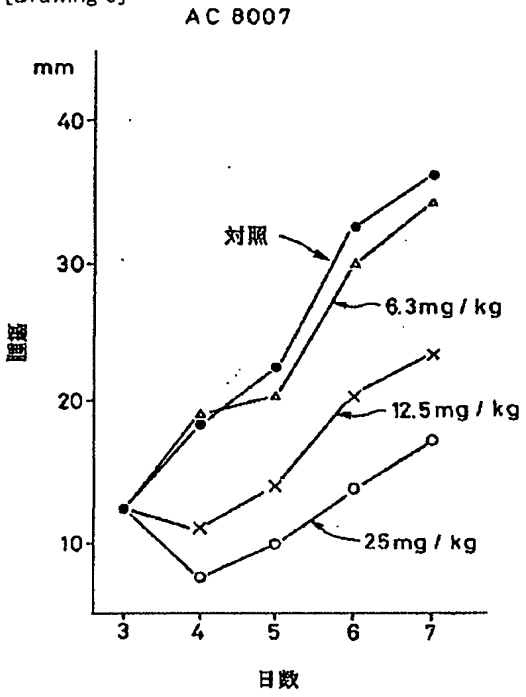
[Drawing 4]



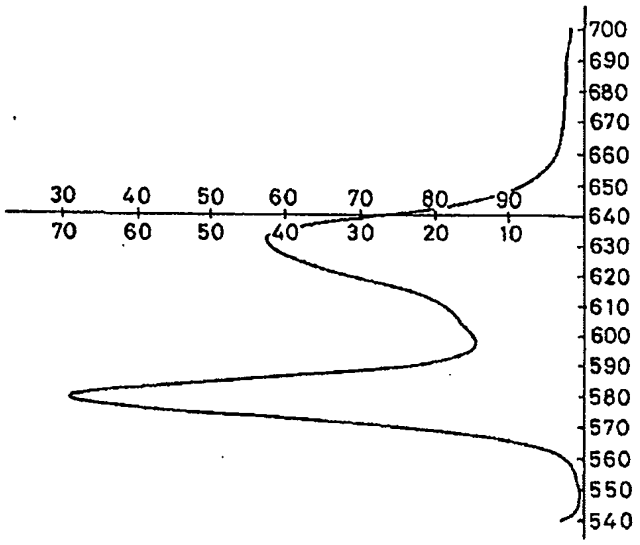
[Drawing 5]



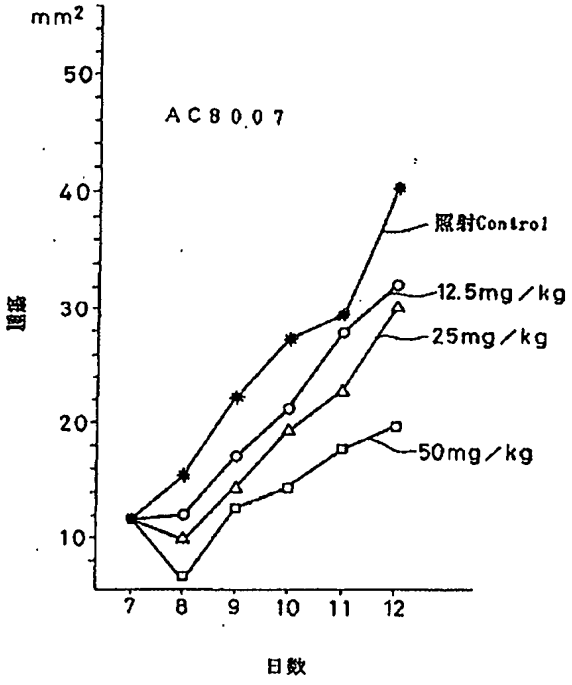
[Drawing 6]



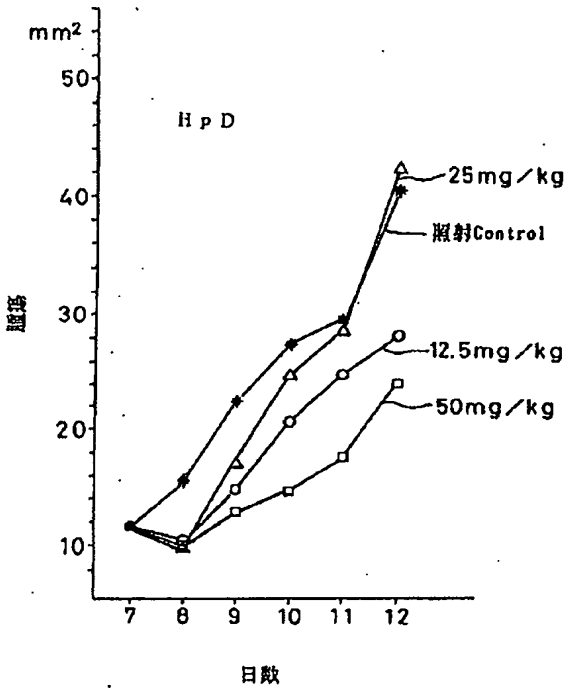
[Drawing 7]



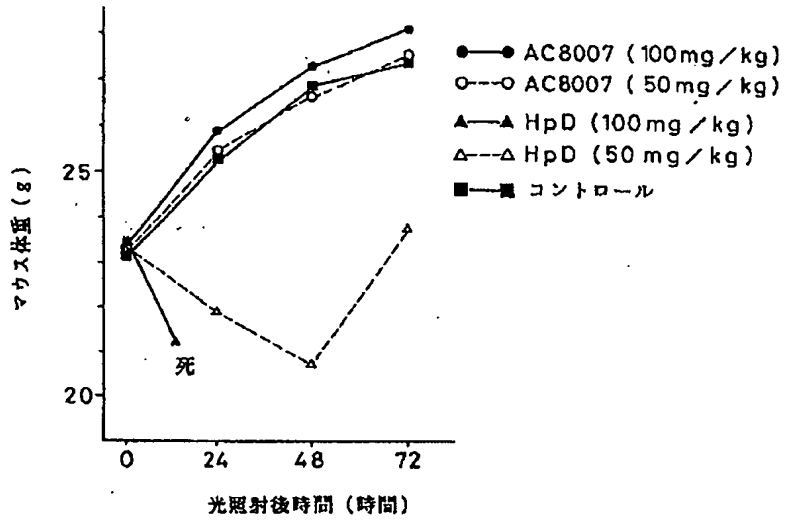
[Drawing 8]



[Drawing 9]



[Drawing 10]



[Translation done.]